REMARKS

With entry of this amendment, claims 1-58 are pending in the application. Claims 31-45 and 56-57 are withdrawn from consideration as noted below.

Election/Restrictions

The Office has presented a Restriction Requirement in the application specifying the following allegedly separate and distinct inventions:

I. Claims 1-30, 46-55 and 58, drawn to a chimeric PIV, classified in class 424, subclass 211.1.

II. Claims 31-45, drawn to a method for stimulating the immune system, classified in class 424, subclass 93.2.

III. Claims 56-57, drawn to a method for producing a chimeric PIV, classified in class 435, subclass 69.1.

During a telephone conversation with Examiner Brown on July 31, 2001, Applicants' representative Jeffrey King made a provisional election with traverse to prosecute the invention of Group I, claims 1-30, 46-55, and 58. The provisional election is hereby affirmed. By this election, no representations are made concerning the merits of the Restriction Requirement with respect to the possible existence of multiple distinct inventions among the originally presented claims. By this election, claims 31-45 and 56-57 are withdrawn from further consideration as being drawn to a non-elected invention.

Drawings

Applicants acknowledge the Draftsperson's objection to the drawings and will submit Formal Drawings resolving these objections in due course.

Specification

The disclosure is objected to because of the following informalities.

Page 113 is missing a printed page number. This informality has been corrected.

The Office further asserts that "amino acid positions throughout the specification must be referred to by a SEQ ID NO, for example, see page 48." Applicants respectfully traverse this formal requirement.

Applicants submit that the Sequence Rules do not require identification of "amino acid positions" by a SEQ ID NO. In accordance with 37 CFR § 1.821(a), identification of individual amino acids or nucleotides in the specification, such as by the designation "Tyr942, Leu992, or Thr1558 of JS cp45" (see, e.g., page 43, line 27 of disclosure), does not invoke the requirement for inclusion of these designates in a separate Sequence Listing. Rather, this requirement is invoked only for "an unbranched sequence of four or more amino acids or an unbranched sequence of ten or more nucleotides."

Applicants' references to individual nucleotides and amino acids in the specification and claims as noted by the Office are therefore believed to be in full compliance with the Sequence Rules. Notably, these references to individual nucleotides and amino acids are further clarified by reference to sequences provided in priority patent applications and/or publications incorporated by reference in the disclosure and/or constituting sequence information that is widely known in the art and therefore not necessary to incorporate in the disclosure.

In view of the foregoing, withdrawal of the stated objection to the specification for alleged non-compliance with the Sequence Rules, is earnestly solicited.

Patentability Under 35 USC § 112

Claims 19, 26-27, 29-30 and 45 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite. In particular, the Office states that the subject claims "recite amino acids positions that must be referred to by a SEQ ID NO." Applicants respectfully traverse.

As noted above, identification of individual amino acids or nucleotides in the specification, (e.g., "Tyr942, Leu992, or Thr1558 of JS cp45" at page 43, line 27), does not invoke a separate Sequence Listing requirement for these designates. This is only required for "an unbranched sequence of four or more amino acids or an unbranched sequence of ten or more nucleotides." With respect to such designates in the claims, 37 CFR § 1.821(d) only requires notation of a SEQ ID NO when the claims recite "a sequence that is set forth in the 'Sequence Listing' . . .." Since identification of individual amino acids or nucleotides is not provided nor required in the Sequence Listing, such identification in the claims is not believed to invoke a requirement for an accompanying identifier "SEQ ID NO:" in the text of the claims. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 29-30 are rejected for reciting, respectively, a chimeric PIV "which is a virus", and "which is a subviral particle". Applicants also respectfully traverse this ground of rejection, but note that the rejection is rendered moot by the clarifying amendments and remarks relating to the subject claims, presented herein.

In particular, the alleged lack of clarity and formal error in limiting dependent claims have been resolved by amendment of claim 29 herein to recite that the claimed PIV is "a complete virus."

As is clearly denoted in the specification and original claims, the "parainfluenza virus (PIV)" of claim 1, from which claims 29-30 depend, is directed to an isolated infectious viral particle which comprises, at a minimum, the N, P, and L proteins. As such, this basic virus particle is viable and infectious without the inclusion of non-essential components of a "complete" PIV. In contrast, as is also disclosed in the specification, it is within the scope of the invention to provide recombinant PIVs that comprise essentially complete viruses, i.e., with all essential viral components and further including non-essential components as found in a complete, e.g., wild-type, PIV. Clearly representative of these teachings, the specification teaches that certain non-essential genes and/or genome segments, for example the C, D and V genes, can be ablated or

otherwise modified to yield desired effects on virulence, pathogenesis, immunogenicity and other phenotypic characters.

Thus, there is no lack of clarity of basis for objection to form in the instant claims 29 and 30, considering that claim 29 now more clearly recited a "complete virus" as a dependent species within the scope of claim 1. Consistent with this terminology, the invention encompasses both complete viral particles and "subviral" particles, the latter of which are disclosed in the specification and will be understood by the skilled artisan to encompass viable, infectious recombinant PIV engineered to lack one or more non-essential components of a "complete" PIV.

Considering the foregoing evidence and remarks, Applicants respectfully submit that the subject matter of claims 29 and 30 is conveyed with sufficient clarity, and therefore request that the rejection of claim these claims under 35 U.S.C. 112, second paragraph, be withdrawn.

Double Patenting

Claims 1-10, 12, 19-30, 46-50, 53 and 54 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-6, 8- 12, 15-16, 18-22, 24-26, 34-39 and 40 of copending Application No. 09/458,813. Applicants note that this is a provisional double patenting rejection and will respond as appropriate upon indication of allowable subject matter in one or the other allegedly conflicting applications.

Patentability Under 35 USC § 102

Claims 1-10, 12, 19-23, 25, 28-29, 46-50 and 53-55 are rejected under 35 U.S.C. 102(e) as being anticipated by Belshe et al (US Patent 5,869,036).

The Office characterizes the subject claims as drawn to an isolated infectious chimeric PIV comprising N protein, P protein, L protein, and a human PIV vector genome or antigenome that is modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a second, antigenically distinct HPIV. The claims encompass different combinations of HPIV1, 2 and 3 domains, such as HN and F proteins. RSV and BIV are also stated to be incorporated as a heterologous gene segment into the vector. The Office also characterizes the claims as being directed to nucleic acids encoding the chimeric PIV and immunogenic compositions comprising the chimeric PIV.

Belshe et al. is cited for teaching:

> an isolated cp-45 hybrid virus (a derivative of HPIV-3 JS) which is suitable for use as a vaccine in humans and animals comprising nucleic acid encoding nucleocapsid protein, phosphoprotein, at least one surface antigen of a target virus, and large polymerase protein, see columns 2-3. The target virus must have enveloped and have one or more surface antigens or surface glycoproteins (HN and F are surface glycoproteins), such as HPIV-1, HPIV-2 and RSV. Belshe et al disclose that the gene sequence which encodes the surface glycoproteins of the target virus may be substituted for the corresponding sequence in the cp45 genome which codes for the HN and F proteins, to result in a chimeric genome, see columns 8-9. Bovine RSV and cattle HPIV- are also included within the scope of Belshe et al. Attenuating mutations are introduced into the L segment as well as other proteins, see column 5, lines 42-67 and column 6, lines 1-3. Belshe et al disclose the use of their chimeric PIV in a vaccine, or immunogenic composition, comprising a physiologically acceptable carrier, see column 2, lines 32-33.

Therefore, the claimed invention is anticipated by Belshe et al.

Applicants respectfully traverse the stated grounds for rejection and submit that the Belshe et al. reference neither teaches nor suggests the subject matter of the claimed invention.

Proper application of the Belshe et al. patent as an allegedly anticipatory reference under 35 U.S.C. § 102 places a direct burden on the Office to demonstrate that the reference discloses each and every element and limitation of the claimed invention.

> The factual determination of anticipation requires the disclosure in a single reference of every element of the claimed invention. . . .[I]t is incumbent upon the examiner to identify wherein each and every facet of the claimed invention is disclosed in the applied reference.

Ex Parte Levy, 17 USPQ2d, 1461, 1462 (Bd.Pat.App.Int. 1990) (emphasis supplied, citations omitted).

In addition, any reference that is relied upon by the Office as anticipatory must fulfill all of the written description and enablement requirements of 35 U.S.C. § 112 commensurate with the rejected claims.

> The standard for anticipation by patenting is the same one of a full enabling disclosure that applies to printed publications, i.e., it must disclose the invention in such full, clear and exact terms as to enable any person skilled in the art to which the invention relates to practice it.

Electronucleonics Laboratories, Inc. et al. v. Abbot Laboratories, 214 USPQ 139, 147 (N.D. Ill. 1981) (underscore added, citations omitted).

As further explained by the Federal Circuit in *In re Donohue*, 226 USPQ 619, (Fed. Cir. 1985).

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it.
>
> ***
>
> [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. (emphasis supplied, citing In re Borst, 45 USPQ 544, 557 (CCPA 1965), *cert. den.* 382 U.S. 973, 148, USPQ 771 (1966).

In the instant case, the Belshe et al. reference provides neither a written description nor an enabling disclosure of the subject matter set forth in Applicants' claims— sufficient to place this subject matter into the hands of the public. For the same reasons, the Belshe et al. reference fails to render the instant claims obvious within the meaning of 35 U.S.C. § 103.

In contrast to Applicants' disclosure setting forth successful recovery of specific, recombinant chimeric and attenuated PIV vaccine candidates, the Belshe et al. reference contains no working example of any such materials or methods as are set forth in Applicants' claims. Instead, the instant rejection is evidently founded on prophetic content provided by the Belshe et al. patent.

The Belshe et al. specification provides a limited description relating to the use of a plasmid expressing a wild type PIV3 L protein to enhance the replication of a JScp45 virus at a restrictive temperature of 39.5°C. This limited disclosure is offered as a basis for speculation by Belshe et al. that the L gene of cp45 possesses mutations that might be useful in a recombinant PIV vaccine virus derived from cDNA.

However, the virus recovered by Belshe et al. after complementation at the restrictive temperature was not changed or modified in any manner contemplated by Applicants' disclosure. No cDNA constructs were designed and produced from which PIV3 wild type viruses could be recovered, and certainly no new constructs or recombinant viruses bearing a chimeric genome or antigenome, and/or specific, attenuating mutations were produced.

Resolving these complex deficiencies in the art was critical to the discovery and reduction to practice of Applicants' claimed invention. At the same time, the absence of such disclosure in the Belshe et al. reference negates any "reasonable expectation for success" to achieve Applicants' invention. This is especially clear when the "particular results" achieved by Applicants are considered, namely that it was shown to be possible to construct recombinant chimeric PIV vaccine candidates from cDNA that are suitably attenuated and immunogenic for development as vaccine candidates.

The principal teachings of Belshe et al. relied upon by the Office are presented, e.g., at columns 2-3 and 8-9 of the Belshe et al. specification, and particularly by the following passage:

> The observation that the temperature sensitive phenotype and the associated attenuation of the cp45 strain of HPIV-3 is caused by a variation in the L gene of cp45 enables a method for producing vaccines for other viruses, such other viruses referred to herein as target viruses. Target viruses include any enveloped virus that has one or more surface antigen. (underscore added)

Thus, Belshe et al. speculate broadly, from the simple result of identifying the L gene of HPIV-3 as allegedly "associated" with attenuation of a biologically derived vaccine candidate (JScp45), that it would be possible to make all manner of attenuated chimeric viruses from "any enveloped virus" using the L gene of JScp45 as an attenuation vehicle.

Examining the teachings of Belshe et al. in further detail, it is noteworthy that the association of the L gene of JS cp45 with attenuation, presented by Belshe et al. as the principal tool for making attenuated "hybrid" viruses, was described previously by Stokes et al. 1993 (of record). Likewise, the properties of JS cp45 as a candidate vaccine virus for primates were previously described by Hall et al. 1993 (of record). The independent contribution of the L gene mutations to the attenuation phenotype of PIV, or to the ts phenotype of the virus, are not described in the Belshe reference or elsewhere in the art of record. This is because the Belshe et al. specification fails to describe or enable recovery of any virus from cDNA, much less specific, chimeric and/or attenuated PIV vaccine candidates as described in Applicants' specification.

The Belshe et al. reference speculates, but presents no findings, that a recombinant virus with one or more of the cp45 L mutations might serve as a useful vaccine candidate against PIV3. However, in order to determine whether such a recombinant virus contains the complex set of biological properties necessary for development of a live attenuated virus vaccine (including viability, attenuation, immunogenic, and protective efficacy), it is first necessary to generate recombinant

viruses using cDNA technology as disclosed by Applicants. It is further necessary to demonstrate that the phenotypic effect of any desired cp45 mutation, for example a ts mutation identified in L, can be segregated from complementary or interactive effects of other cp45 mutations. Lastly, it is critical for validating the speculative teachings of Belshe et al. that any mutations thus identified and segregated into a viable recombinant vaccine candidate are in fact attenuating and that such attenuation can be balanced sufficiently to yield a protective immune response in susceptible hosts.

The simple studies of Belshe et al. involved complementation of replication for a cp45 virus using a wild type L plasmid. These studies were only conducted *in vitro* using tissue culture cells, and were not validated by parallel studies *in vivo*. In this context, it was quite possible that recombinant viruses incorporating one or more of the three "temperature sensitive" (ts) mutations in the cp45 L gene mutations would not be attenuating (att) *in vivo*. In particular, a finding that replication of cp45 may be complemented by wild type L protein in tissue culture cells is not clearly predictive that a virus bearing one or more of these mutations would be attenuated *in vivo*. This correlative deficiency is apparent from the following considerations.

As an initial point, it is known that entire classes of viruses called "temperature-dependent hose range (td-hr) mutants" may be ts on one tissue but not on other tissue culture cells. These td-hr mutants are not necessarily attenuated *in vivo* (see Snyder et al., Virus Research 15:69-84, 1990 and Shimizu et al., Virology 124:35-44, 1983—copy to be provided under separate cover for consideration and entry in the record). As described in Snyder et al., an exemplary mutant (clone 143-1) of influenza virus was shown to be highly ts in tissue culture cells, but was not significantly attenuated *in vivo*. Additional findings by Shimizu et al. indicate that such td-hr mutants are common and are found in many different complementation groups of the influenza virus (i.e., they are present in many different genes of the virus).

The Belshe et al. reference does not demonstrate whether any of the contemplated ts mutations in the L gene of cp45 belong in the td-hr class of mutations or in the other

class of ts mutations whose replication is effected by the temperature present in the host animal. In view of this deficiency, the simple description of a complementation phenotype for a group of multiple, unsegregated mutations in a complete gene *in vitro* does not serve as a reliable indicator of attenuation *in vivo*.

As a second point relating to the reliability of teachings by Belshe et al., the reference also fails to describe specific levels of temperature sensitivity and/or attenuation for any virus bearing one or more of the three, specific cp45 L gene mutations disclosed by Applicants. These important properties cannot be reliably translated from an *in vitro* complementation system as described by Belshe et al. to a hypothetical recombinant PIV vaccine candidate *in vivo*. On the contrary, the teachings of the cited reference merely suggest that the cp45 mutations in L make some contribution to the ts phenotype *in vitro* in one cell line. From these limited teachings, it cannot be reliably predicted what level of temperature sensitivity and/or attenuation any recombinant virus with one or more of the L gene mutations of cp45 may exhibit. For example, if the contribution of the set of cp45 L mutations to the overall level of temperature sensitivity of cp45 was small, and a virus bearing all three cp45 mutations in L was restricted at 39.5°C (the only temperature tested by Belshe et al.) but not at 37°C, such a virus may not be attenuated at all in a host with a 37°C body temperature. Thus, the disclosure of Belshe et al. does not demonstrate any properties of the cp45 L gene mutations that are necessary for designing a recombinant virus that has useful properties for vaccine use.

The complex effects and interactions of mutations in the PIV3 cp45 virus that determine its level of temperature sensitivity and attenuation, as demonstrated in the present application, clearly show that predictions of *in vivo* properties of individual and collective mutations in recombinant PIVs cannot be reliably made based on *in vitro* complementation studies as presented by Belshe et al. For example, prior to Applicants' invention it was not predictable that the temperature sensitive phenotypes of the cp45 L gene mutations recovered in recombinant PIVs engineered from cDNA would not be additive. However, as revealed in the instant specification, the assembly of cp45 992 and 1558 mutations generates a recombinant virus bearing two "ts" mutations that is less

temperature sensitive than a recombinant bearing either single mutant. It is only with the benefit of Applicants' invention that these unexpected effects were discerned and recognized as useful tools for calibrating attenuation and immunogenicity in recombinant PIV vaccine candidates.

In relation to the foregoing points, the Belshe reference erroneously concludes that a temperature sensitive phenotype accurately predicts the presence of an attenuation phenotype in a recombinant PIV. Applicants' disclosure reveals the flawed nature of this conclusion. In this context, the instant specification teaches that the rcp45 3'N recombinant was ts but not attenuated and, conversely, the rcp45 C and rcp45 F recombinants were attenuated and not ts. Furthermore, r942/992, which exhibited a level of temperature sensitivity comparable to that of cp45 virus, was overattenuated *in vivo*. Conversely, r992/1558 was much less attenuated that cp45. These unexpected findings underscore the deficiencies of the Belshe et al. patent— which fails to identify specific properties determined by individual cp45 L gene mutations, much less to reliably predict combinatorial phenotypes specified by sets of mutations incorporated within novel recombinant vaccine candidates and analyzed *in vivo*. Only through the use of Applicants' successful cDNA recovery system could these unexpected effects be determined and harnessed for use within the claimed methods and compositions.

Thus, the principal disclosure of Belshe et al. that is purported to render construction of chimeric PIV and other "hybrid" viruses possible (i.e., the attenuating role of the L gene of JScp45) in no way teaches or suggests the presently claimed subject matter. This is even more apparent when one considers the complexities and unpredictable barriers involved in the construction of chimeric PIV viruses from cDNA. As noted above, the studies of Belshe et al. involved simple complementation of replication for a cp45 virus using a wild type L plasmid. No chimeric cDNA constructs were described in any form that would be commensurate with the written description and enablement requirements of 35 U.S.C. § 112. No specific guidance is provided to enable any kind of cDNA recovery of PIV, much less recovery of a viable, attenuated and infectious chimeric PIV, as provided by Applicants. The Office is respectfully requested

to reconsider these issues as they relate to the interpretation of Belshe et al. as a basis for rejection of the instant claims.

The speculative and non-enabling teachings of Belshe et al. are believed by Applicants to be apparent from the record, without need for further elaboration. This conclusion is facially apparent from the foregoing discussion, and underscored by the diversity of viral "targets" contemplated by Belshe et al. for constructing "hybrid" viruses, as indicated by the following passage:

> Hence, in addition to related enveloped, negative-sense, single-stranded RNA viruses such as human parainfluenza virus type 1 (HPIV-1), human parainfluenza virus type 2 (HPIV-2), respiratory syncytial virus (RSV), human influenza virus type A, human influenza virus type B, and measles viruses, target viruses would also include other enveloped viruses, such as paramyxoviruses, orthomyxoviruses, retroviruses (e.g. human immunodeficiency viruses HIV-GP120 and HIV-GP41), arenaviruses, coronaviruses, bunyaviruses, rhabdoviruses, togaviruses, herpesviruses, poxviruses and hepadnaviruses. Preferable target viruses include enveloped viruses which reproduce in the cytoplasm. The target virus of the present invention may be specific to humans, specific to animals or common to both animals and humans. Bovine RSV and cattle HPIV-3 (shipping fever virus) are typical animal viruses included within the scope of the present invention. [Col. 8, lines 42-58, underscore added.]

In contrast to these broad, prophetic teachings, Applicants specification provides detailed description and guidance, as well as representative working examples, in a manner that is fully commensurate with the scope of claims presented for review. The course of examination in this application indicates that the Office has acknowledged the completeness of these teachings. On the contrary, the foregoing discussion clearly evinces that Belshe et al. does not provide such a fully descriptive and enabling disclosure of the claimed subject matter, and therefore neither teaches nor suggests Applicants' methods and compositions.

Patentability Under 35 USC § 103

Claims 1-30 and 46-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belshe et al in view of Collins et al (US Patent 6,264,957) and Klein et al (W093/14207).

The teachings of Belshe et al. are relied upon by the Office as described above. Belshe et al. is further cited for allegedly teaching "attenuating mutations in the HPIV3 L protein wherein leucine is substituted for phenylalanine". The Office notes that Belshe et al. does not teach "where the heterologous gene segments are added, a subviral particle, an attenuating mutation at position 456 of HPIV3 L protein stabilized by multiple nucleotide changes in a codon specifying the mutation." It is also stated by the Office that Belshe et al. "do not teach an immunogenic composition dosage and route of administration."

Collins et al. is cited for allegedly teaching "RSV vaccines comprising subviral particles."

Klein et al. is cited for allegedly teaching "a multimeric hybrid gene, comprising RSV (G or F protein) and HPIV (F or HN protein), and combinations of these proteins such as F proteins from both PIV3 and RSV". In addition Klein et al. is cited for teaching "a vaccine formulated for administration intranasally."

On the basis of these combined disclosures, the Office asserts that one of ordinary skill in the art "would have been motivated to modify the chimeric PIV of Belshe et al. by substituting subviral particles because it was known in the art at the time of the invention that subviral particles are effective in vaccine compositions as taught by Collins et al." Furthermore, the Office contends that "[o]ne of ordinary skill would know the dosage required to elicit an immune response and would have been motivated to make the modifications of dosage and administration in order to achieve the maximum immune response." It is also asserted by the Office that a person skilled in the art "would also

know where to add the heterologous gene segment given the well-known art of recombination and would have been motivated to incorporate the segment in such a way as to ensure its expression and stability" Finally, it is asserted by the Office that "Belshe et al. teach the method of incorporating the heterologous (target gene clone) segment by ligation into the PIV clone."

Applicants respectfully traverse the foregoing grounds of rejection and submit that the invention of claims 1-6, 8-26, 34-45, 47 and 51-52 is neither disclosed nor suggested by the combined teachings of Belshe et al., Collins et al. and Klein et al. viewed for what they teach as a whole.

The fundamental grounds stated in support of the instant rejection are based on the limited teachings of Belshe et al., as discussed above. In this regard, the Office contends that it would have been obvious to "modify the chimeric PIV of Belshe et al." using the secondary teachings of Collins et al. and Klein et al.

However, as noted above, Belshe et al. do not disclose a "PIV clone" that would be a potential subject for modification in accordance with the Office's proposal. On the contrary, as discussed in detail in the preceding section, Belshe et al. neither describes nor enables such a recombinant PIV clone. It is even clearer from the preceding discussion that this primary reference does not teach nor suggest methods and compositions that would be useful for generating live, attenuated chimeric PIV according to Applicants' invention. Instead, the Belshe et al. reference provides, at best, an invitation to experiment toward the production of "hybrid" viruses selected from "any enveloped virus that has one or more surface antigen."

For these reasons, the Belshe et al. reference is notably deficient as a primary reference that would satisfy the Office's interpretation. The secondary teachings of Collins et al. (relating to "RSV vaccines comprising subviral particles", and of Klein et al., relating to "a multimeric hybrid gene" between RSV and HPIV glycoproteins, and "a vaccine formulated for administration intranasally"), clearly fail to rectify this primary deficiency. In particular, these secondary teachings do not supplement the teachings of

Belshe et al. in a direction or manner that would provide the requisite "reasonable expectation of success" for producing live, attenuated, chimeric PIV viruses or subviral particles for development as vaccine candidates as disclosed and claimed by Applicants.

Accordingly, the rejection of claims 1-6, 8-26, 34-45, 47 and 51-52 under 35 U.S.C. 103(a) over Belshe et al. in view of Collins et al. and Klein et al. is believed to be overcome.

CONCLUSION

In view of the foregoing, Applicants believe that all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes that a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-332-1380.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Date: February 13, 2002

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. An isolated infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a human PIV (HPIV) vector genome or antigenome that is modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a second, antigenically distinct HPIV.

2. The chimeric PIV of claim 1, wherein one or more heterologous genome segment(s) of the second, antigenically distinct HPIV encoding said one or more antigenic domains, fragments, or epitopes is/are substituted within the HPIV vector genome or antigenome to encode said chimeric glycoprotein.

3. The chimeric PIV of claim 2, wherein said one or more heterologous genome segment(s) encode(s) one or more glycoprotein ectodomain(s) substituted for one or more corresponding glycoprotein ectodomain(s) in the vector genome or antigenome.

4. The chimeric PIV of claim 2, wherein heterologous genome segments encoding both a glycoprotein ectodomain and transmembrane region are substituted for counterpart glycoprotein ecto- and transmembrane domains in the vector genome or antigenome.

5. The chimeric PIV of claim 1, wherein said chimeric glycoprotein is selected from HPIV HN or F glycoproteins.

6. The chimeric PIV of claim 1, wherein the (HPIV) vector genome or antigenome is modified to encode multiple chimeric glycoproteins.

7. The chimeric PIV of claim 1, wherein the HPIV vector genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct HPIV is selected from HPIV1 or HPIV2.

8. The chimeric PIV of claim 7, wherein the HPIV vector genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct HPIV is HPIV2.

9. The chimeric PIV of claim 8, wherein one or more glycoprotein ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein ectodomain(s) in the HPIV3 vector genome or antigenome.

10. The chimeric PIV of claim 9, wherein both glycoprotein ectodomain(s) of HPIV2 HN and F glycoproteins are substituted for corresponding HN and F glycoprotein ectodomains in the HPIV3 vector genome or antigenome.

11. The chimeric PIV of claim 10, which is rPIV3-2TM.

12. The chimeric PIV of claim 10, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp*45.

13. The chimeric PIV of claim 12, which is rPIV3-2TM*cp*45

14. The chimeric PIV of claim 8, wherein PIV2 ectodomain and transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more corresponding PIV3 cytoplasmic tail region(s).

15. The chimeric PIV of claim 14, wherein ectodomain and transmembrane regions of both PIV2 HN and F glycoproteins are fused to corresponding PIV3 HN and F cytoplasmic tail regions.

16. The chimeric PIV of claim 15, which is rPIV3-2CT.

17. The chimeric PIV of claim 16, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp*45.

18. The chimeric PIV of claim 15, which is rPIV3-2CT*cp*45.

19. The chimeric PIV of claim 1, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp*45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp*45; in the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp*45, in the C protein at a position corresponding to Ile96 of JS *cp*45, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp*45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp*45

20. The chimeric PIV of claim 1, wherein a plurality of heterologous genes or genome segments encoding antigenic determinants of multiple heterologous PIVs are added to or incorporated within the partial or complete HPIV vector genome or antigenome.

21. The chimeric PIV of claim 20, wherein said plurality of heterologous genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and are added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

22. The chimeric PIV of claim 20, wherein the chimeric genome or antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or epitopes from two or more different HPIVs.

23. The chimeric PIV of claim 1, wherein the chimeric PIV genome or antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory element from a bovine PIV3 (BPIV3).

24. The chimeric PIV of claim 1, wherein the chimeric PIV genome or antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide sequence(s).

25. The chimeric PIV of claim 1, wherein the chimeric genome or antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or epitopes from both HPIV3 JS and HPIV1 or HPIV2.

26. The chimeric PIV of claim 1, wherein the chimeric genome or antigenome is modified by introduction of an attenuating mutation involving an amino acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

27. The chimeric PIV of claim 26, wherein phenylalanine at position 456 of the HPIV3 L protein is substituted by leucine.

28. The chimeric PIV of claim 1, wherein the chimeric genome or antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or immunogenic domain(s), fragment(s), or epitope(s) thereof.

29. (Amended) The chimeric PIV of claim 1 which is a complete virus.

30. The chimeric PIV of claim 1 which is a subviral particle.

31. (Withdrawn) A method for stimulating the immune system of an individual to induce protection against PIV which comprises administering to the individual an immunologically sufficient amount of the chimeric PIV of claim 1 combined with a physiologically acceptable carrier.

32. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered in a dose of 10^3 to 10^7 PFU.

33. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered to the upper respiratory tract.

34. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered by spray, droplet or aerosol.

35. (Withdrawn) The method of claim 31, wherein the vector genome or antigenome is of human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV1 and/or HPIV2.

36. (Withdrawn) The method of claim 31, wherein the chimeric PIV elicits a polyspecific immune response against multiple human PIVs.

37. (Withdrawn) The method of claim 31, wherein a first, chimeric PIV and a second PIV are administered sequentially or simultaneously to elicit a polyspecific immune response.

38. (Withdrawn) The method of claim 37, wherein the second PIV is a second, chimeric . PIV according to claim 1.

39. (Withdrawn) The method of claim 37, wherein the first, chimeric PIV and second PIV are adminstered simultaneously in a mixture.

40. (Withdrawn) The method of claim 37, wherein the first and second chimeric PIVs are bear the same or different heterologous antigenic determinant(s).

41. (Withdrawn) The method of claim 37, wherein the first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits an immune response against HPIV1 or HPIV2.

42. (Withdrawn) The method of claim 37, wherein the second chimeric PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of respiratory syncytial virus (RSV).

43. (Withdrawn) The method of claim 42, wherein both the first and second chimeric PIVs elicit an immune response against RSV.

44. (Withdrawn) The method of claim 43, wherein the first chimeric PIV is adminstered initially in a vaccination protocol and the second chimeric PIV is

adminstered subsequently in the vaccination protocol to provide initial immunization against HPIV3 and secondary immunization against HPIV1 or HPIV2 and to provide initial and secondary, booster immunization against RSV.

45. (Withdrawn) The method of claim 37, wherein the first, chimeric PIV incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS cp45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position corresponding to Ile96 of JS cp45, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

46. An immunogenic composition to elicit an immune response against PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a physiologically acceptable carrier.

47. The immunogenic composition of claim 46, formulated in a dose of 10^3 to 10^7 PFU.

48. The immunogenic composition of claim 46, formulated for administration to the upper respiratory tract by spray, droplet or aerosol.

49. The immunogenic composition of claim 46, wherein the chimeric PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2 and HPIV3.

50. The immunogenic composition of claim 46, wherein the chimeric PIV elicits an immune response against HPIV3 and another virus selected from HPIV1, HPIV2, and respiratory syncytial virus (RSV).

51. The immunogenic composition of claim 46, further comprising a second, chimeric PIV according to claim 1.

52. The immunogenic composition of claim 51, wherein the first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second chimeric PIVs elicit an immune response against RSV.

53. An isolated polynucleotide comprising a chimeric PIV genome or antigenome which includes a human PIV (HPIV) vector genome or antigenome modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a second, antigenically distinct HPIV.

54. The isolated polynucleotide of claim 53, wherein one or more heterologous genome segment(s) encoding the antigenic domains, fragments, or epitopes of said second, antigenically distinct HPIV is/are substituted for one or more counterpart genome segment(s) in the HPIV vector genome or antigenome.

55. The isolated polynucleotide of claim 53, wherein, the chimeric genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS *cp*45.

56. (Withdrawn) A method for producing an infectious attenuated chimeric PIV particle from one or more isolated polynucleotide molecules encoding said PIV, comprising:

expressing in a cell or cell-free lysate an expression vector comprising an isolated polynucleotide comprising a vector genome or antigenome modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a second, antigenically distinct HPIV, and PIV N, P, and L proteins.

57. (Withdrawn) The method of claim 56, wherein the chimeric PIV genome or antigenome and the N, P, and L proteins are expressed by two or more different expression vectors.

58. An expression vector comprising an operably linked transcriptional promoter, a polynucleotide sequence which includes a vector genome or antigenome modified to encode a chimeric glycoprotein incorporating one or more heterologous antigenic domains, fragments, or epitopes of a second, antigenically distinct HPIV, and a transcriptional terminator.